Antimicrobial, Physicochemical, Mechanical, and Barrier Properties of Tapioca Starch Films Incorporated with Eucalyptus Extract

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Abstract: Starch is found in abundance in nature and it is one of the raw materials used for food packaging because of the low price, biodegradability, good mechanical and barrier properties. The recycling ability of coating materials was significantly increased by using edible films and coating in comparison to traditional packaging and it could be an alternative for synthetic films. In this research, the effect of eucalyptus extract (Aqueous Extract) was investigated on tapioca starch films. Tapioca starch films were prepared by casting method with addition of eucalyptus extract and a mixture of sorbitol/glycerol (weight ratio of 3 to 1) as plasticizers. Eucalyptus extract incorporated to the tapioca starch films were dried at different concentrations (0, 15, 25, and 35 of total solid) under controlled conditions. Physicochemical properties such as water absorption capacity (WAC), water vapor permeability (WVP) and mechanical properties of the films were evaluated. Results showed that by increasing the concentration of eucalyptus extract, tensile strength was increased from 20.60 to 15.68 (MPa), also elongation was increased from 19.31 to 23.57 (%) at break and Young’s modulus was decreased from 800.31 to 500.32 (MPa). Also incorporation of eucalyptus extract in the structure of biopolymer increased permeability of water vapor of starch films. Tapioca starch films incorporated with eucalyptus extract exhibited excellent antimicrobial activity against E. Coli. In summary, eucalyptus extract improves functional properties of tapioca starch films and this types of films can be used in food packaging.

Keywords: Eucalyptus extract, Tapioca Starch film, WVP, Antimicrobial, Mechanical Properties

INTRODUCTION

Replacing the new packaging system can be the main target for all consumers and manufactures. Although replacing all biodegradable plastics with synthetic material for long life food is possible [1]. There are increasing public concerns regarding the environmental pollution caused by excessive waste from packaging materials. Indeed food packaging is one of the main contributors to this waste and although some materials can be recovered and recycled. Many synthetic polymer based materials end up in landfill or the environment. Edible and biodegradable natural-polymer films offer alternatives to conventional packaging due to their excellent biodegradability, biocompatibility and edibility, and the range of their potential applications [2]. Carbohydrate-based edible films, which have good film-forming ability due to their unique colloidal properties, are particularly attractive. Starch is the most commonly used raw
material in agriculture due to its relatively low cost, wide availability and ease of handling [3]. Tapioca starch is differentiated from other starches by its low level of residual materials (fat, protein, ash) lower amylose content than for other amylose-containing starches, and high molecular weights of amylose and amylpectin. The small amount of phosphorus in tapioca starch is partially removable [4] and, therefore, not bound as the phosphate ester as in potato starch. The very low protein and lipid content is an important factor which differentiates tapioca starch from the cereal starches. Typically, cassava starch contains 17–20% amylose. Unlike corn (0–70% content) and rice (0–40% amylose content), no significant variation of amylose content has been found in cassava starch.

Despite recent achievements in food safety technologies, epidemiological studies have demonstrated that the number of diseases caused by food-borne pathogenic microorganisms has increased in recent years [5]. Therefore, one of the major challenges for food technologists is the design of active food packaging and in particular, antimicrobial packaging. Essential oils extracted from plants or spices are rich sources of biologically active compounds such as terpenoids and phenolic acids. The ability of plant essential oils to protect foods against pathogenic microorganisms has been reported by several researchers [6].

_Eucalyptus_ is aromatic and medicinal plants belonging to the Myrtle family. An essential oil extracted from eucalyptus leaves contains compounds that are powerful natural disinfectants and can be toxic in large quantities [7].

The use of herbal extract has become very attractive for several applications in the food industry, particularly due to the successful results obtained so far. However, further studies are necessary on the potential of each essential oil. Therefore, the aim of this research was to develop an antimicrobial film based on tapioca starch and _Eucalyptus_ extract; and to study the effect of the incorporation of _Eucalyptus_ extract on the antimicrobial, physical, mechanical, barrier, and optical properties of films to examine their potential applications as active-packaging material.

**MATERIALS AND METHODS**

Cassava starch was purchased from SIM Company (Penang, Malaysia). Glycerol was purchased from Merck. For the antimicrobial assay, _E. Coli_ culture was supplied from the Islamic Azad University culture collection center (Damghan, Iran). Stock cultures of the studied bacteria were grown in MHB at 30 °C for 24 h before the tests.

**Film preparation**

Tapioca starch dispersion 4% (w/w) was heated to 90°C and held at this temperature for 45 min to complete the gelatinization. A 3:1 mixture of sorbitol-glycerol at 40% (w/w of starch) was added as plasticizers; the choice of the plasticizers was based on the previous research by Abdorreza and his colleagues [8]. Starch dispersions were heated to 85±5 °C and held for 45 min to allow gelatinization. Upon completion of starch gelatinization, the solution was cooled to room temperature. 15, 25, and 35% of _Eucalyptus_ extract concentrations were added to dispersions and mixed for 1 hour. A portion (92 g) of the dispersion was cast on Perspex plates fitted with rims around the edge to yield a 16×16 cm² film-forming area. Films were dried under controlled conditions in a humidity chamber (25 °C and 50% RH). Control films were prepared similarly but without addition of _Eucalyptus_ extract. Dried films were peeled and stored at 23 ± 2 °C and 50 ± 5% relative humidity (RH) until experimentation. All films (including control) were prepared in duplicate.
Mechanical Properties
ASTM D882-10 [9] was used with some modifications to determine the mechanical properties under standard conditions [10]. Elongation and tensile strength at break were calculated from the deformation and force data recorded by the software. Eight replications were evaluated for every sample.

Water Absorption Capacity
The water absorption capacity measured as adapted method by Kiatkamjornwong and his colleagues [11]. Starch films first were dried in a P2O5 for one week and then a piece of 2 × 2 cm2 (≈50 mg) of dried films was added to 100 ml of distilled water and allowed to stand for 30 min for swelling. The swollen films were drained and weighted. The amount of water retained by films per dried weight of the films calculated as water absorption capacity.

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\text{Solubility (\%)} = \frac{(\text{Initial dried weight of film} - \text{Final dried weight of film})}{\text{Initial dried weight of film}} \times 100
\]

Antimicrobial assay
An antimicrobial activity test of the films was carried out using the agar diffusion method according to Maizura and his colleagues [12]. Antimicrobial effects of the films were determined by the inhibition zone against S. Aureus on solid media.

Water vapor permeability
The modified gravimetric cup method [14] based on ASTM E96-05 was used to determine the water vapour permeability (WVP) of films. Six samples per treatment were tested.

Oxygen permeability (OP)
Oxygen permeability measurements were performed on films by Mocon Oxtran 2/21 (Minneapolis, USA) comes with Win Perm TM permeability software using the ASTM standard method D3985-05 and equipped with a patented colometric sensor (Coulox®). The films were placed on an aluminum foil mask with an open area of 5 cm2 and were mounted in diffusion cells. Tests were carried out at 25°C temperature, atmospheric pressure, and 50% RH using 21% oxygen as test gas. Transferred oxygen through the films was conducted by the carrier (N2/H2) gas to the colometric sensor. Measurements were carried out on convergent by hour to reach the steady state of oxygen transmission. The permeability coefficients in cc·µm/(m2 day atm) were calculated on the basis of oxygen transmission rate in steady state taking into account the films thickness.

Statistical Analysis
ANOVA and Turkey’s Post Hoc tests were used to compare means of physical, mechanical, thermal, and antimicrobial properties of starch based or gelatin based films at the 5% significance level. Statistical analysis was conducted using GraphPad Prism 6 (GraphPad Software Inc., 2236 Avenida de la Playa, La Jolla, CA 92037, USA).
RESULTS AND DISCUSSIONS

Effects of eucalyptus extract on mechanical properties of tapioca starch films

Figure 1 shows the effect of eucalyptus extract on tensile strength (TS) of tapioca starch films with 40% plasticizer. Addition of eucalyptus extract significantly \((p < 0.05)\) decreased the tensile strength of tapioca starch films. Figures 2 and 3 show other mechanical properties of starch films incorporated with eucalyptus extract: elongation at break and Young’s modulus. Elongation at break has a reverse relation with tensile strength in most cases and Young’s modulus was directly related to tensile strength. Young’s modulus is a measure of the rigidity of the material and improves as nanoparticles. The elasticity of the films is related to interactions of the macromolecules and can be reduced by addition of plasticizer. Decreases in TS and increases in EB are common results of essential-oil incorporation, and have been broadly discussed in research into other biopolymer films. Maizura and his colleagues [12] reported a decrease in the TS of the films prepared from starch-alginate film to which different concentrations of lemongrass oil had been added. They reported that this was due to the effect of the lipid on the starch chains and that the phase rich in polysaccharide had higher TS than the lipid phase.
Effects of eucalyptus extract on water absorption capacity of tapioca starch films

The water absorption capacity of the eucalyptus extract incorporated films is given in Figure 4.

Introducing eucalyptus extract to the tapioca starch matrix has no significant effect on the water absorption capacity of the starch films.

Solubility of tapioca starch films incorporated by eucalyptus extract in water

The solubility of the films is one of the important parameter for packaging films. Solubility in water may be an important factor in defining applications for biopolymer composite films. Most of the biopolymers are sensitive to water. Generally, the effects of additives on the solubility of films depend on the type of compounds and concentrations and their inherent hyrophilicy and hydrophobicity indices. It would be expected that hydrophilic compounds should increase a film’s solubility, whereas hydrophobic compounds should decrease it. By incorporating lipids, crosslinking of the structure or incorporation of nanoparticles sensitivity to water could be decreased [15]. Figure 5 shows the solubility of tapioca starch films in deionized water after 1 h.
Incorporation of *eucalyptus* extract increased the diffusion of water into the structure. So the solubility was significantly increased.

![Figure 5. Effects of eucalyptus extract on water solubility of tapioca starch films. The bars show mean (n=3) ± SD. Different letters on the bars represent the significant difference at 5% level of probability.](image)

**Antimicrobial assay**

Effects of sample films were investigated on the growth of *E. Coli*. The inhibition zone of pure and *eucalyptus* extract incorporated films significantly increased by increasing the *eucalyptus* extract contents. Figure 6 shows the effects of *eucalyptus* extract contents on antimicrobial activity of the different films. Recently the excellent antimicrobial activity of herbal extract and the mechanism of the action against the microorganisms comprehensively investigated [4].

![Figure 6. Effects of eucalyptus extract contents on antimicrobial activity of tapioca starch films. (Inhibition zone = total inhibition area – total film area. The bars shows mean (n=5) ± SD. Different letters on the bars represent the significance difference in 5% level of probability among tapioca starch films.](image)

**Effects of eucalyptus extract on water vapour permeability**

Figure 7 shows the effect of *eucalyptus* extract on the water vapor transmission rate through starch films. As it is expected, due to increasing hydrophilicity of starch films, the WVP of the films significantly decreased. Other researcher also reported same results [12].
Figure 7. Effects of eucalyptus extract on water vapor permeability of tapioca starch films. The bars show mean (n=3) ± SD. Different letters on the bars represent the significant difference at 5% level of probability.

Effects of eucalyptus extract on oxygen permeability of films

Permeability to oxygen and water vapor were performed at standard condition. Figure 8 shows the effects of eucalyptus extract on permeability to oxygen. As it is predictable, permeability decreased significantly. This result is in accordance with permeability to water vapor. Sothornvit (2000) reported that oxygen permeability was dependent on the fluidity of the polymeric branches (moisture content) [16]. Kanatt and his colleagues (2012), in a study investigating polyvinyl alcohol and chitosan films with aqueous extract of mint and pomegranate peel, expressed adding the extract did not affect the permeability characteristics; however, adding chitosan, due to constructing intra-molecular hydrogen bonds with polyvinyl alcohol, restricted the fluidity of the branches and thus reduces the oxygen permeability [17]. Safari and Mohammadi Nafchi also reported the same results about the effects of water extracts of Rosemary on starch films [18].

Figure 8. Effects of eucalyptus extract on oxygen permeability of tapioca starch films. The bars show mean (n=3) ± SD. Different letters on the bars represent the significant difference at 5% level of probability.

CONCLUSION

In this study, we introduced eucalyptus extract to the tapioca starch matrix to fabricate antimicrobial films. Introduction of the eucalyptus extract improved antimicrobial properties of the films made from tapioca starches. Water vapour and oxygen permeability, water absorption capacity,
and water solubility of the films were significantly increased. The results showed that *eucalyptus* extract incorporated starch films have potential applications in active packaging.

**REFERENCES**
